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In re Patent Application of

Plamen Denchev

Application No. 10/764,978

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Examiner: June Hwu

Atty Reference: 205502-9037-US00

"METHOD FOR REPRODUCING CONIFERS BY SOMATIC EMBRYOGENESIS USING GALACTOSE CONTAINING COMPOUNDS AS A CARBON AND ENERGY SOURCE"

I, Jennie Sanderson, hereby certify this correspondence is being deposited with the United States Postal Service in an envelope as "Express Mail Post Office to Addressee," mailing Label Number EV510710975US, addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Jennie Sanderson
Signature

2/16/10
Date

APPEAL BRIEF FILED UNDER 37 C.F.R. §41.31

Mail Stop Appeal Brief - Patents
Commissioner for Patents
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Sir:

Appellant has appealed from the final Office Action dated February 11, 2009 in which the Examiner rejected all of the pending claims. Appellant's attorney timely filed a Notice of Appeal on July 13, 2009. This Appeal Brief is timely filed with a request for five-month extension of time. Please charge the fee required under 37 CFR 1.17(a)(5) to Deposit Account No. 50-1965. Please charge any additional fees or credit any overpayment of fees associated with this Appeal Brief to Deposit Account No. 50-1965.

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REAL PARTY IN INTEREST

The real party in interest is the assignee of record in the case, CellFor Inc.

RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences related to this appeal.

STATUS OF CLAIMS

Claims 1, 5-6, 13, 14, 16-22, 43, 50 and 52-63 are pending in the application. The claims stand variously rejected. Claims 2-4, 7-12, 15, 23-42, 44-49 and 51 are cancelled. Claims 1, 5-6, 13, 14, 16-22, 43, 50 and 52-63 are under examination, were finally rejected in an Office Action dated February 11, 2009 and are appealed.

STATUS OF AMENDMENTS

A final rejection was issued in this case on February 11, 2009. Appellants made no amendments after the final rejection.

SUMMARY OF CLAIMED SUBJECT MATTER

Claim 1 is directed to a method for reproducing coniferous somatic embryos by somatic embryogenesis (see original claim 1). The method includes growing an immature embryogenic culture derived from an explant on a nutrient medium selected from the group consisting of induction medium, maintenance medium and prematuration medium (original claim 1, specification at page 1, lines 25-28, and page 3, lines 10-13). The nutrient medium comprises lactose and an additional sugar (see original claims 4 and 15 and the specification at page 7, lines 5-9). The induction medium is used to induce an explant to form an embryogenic tissue (see specification at page 7, lines 1-5). The maintenance medium is used to grow and maintain the embryogenic culture (see specification at page 4, lines 10-12 and page 8, lines 3-7). The prematuration medium is used to prepare the embryogenic culture for transfer to maturation medium and subsequent development of mature embryos capable of germination (see specification at page 4, lines 7-9; page 3, lines 19-25 and the examples). The coniferous somatic embryos are *Pinus taeda* somatic embryos or hybrids thereof (original claim 9 and page 6, line 17). The maturation medium used to develop the embryos does not contain any auxin or cytokinin (see specification at page 5, lines 11-13, page 8, line 23-page 9, line 1; page 9, lines 15-19 and page 12, lines 3-5).

Claim 43 is directed to a method for reproducing conifers by somatic embryogenesis (see original claim 43). The method includes growing *Pinus taeda* conifer cells on a nutrient medium comprising lactose, an additional sugar, an auxin and a cytokinin to produce an immature embryogenic culture (see original claims 4, 15 and 43 and the specification at page 7, lines 5-9). The embryogenic culture is then transferred to maturation medium that does not contain auxin or cytokinin to obtain mature embryos capable of germination and reproduction of conifers (see specification at page 3, lines 19-25 and the examples).

Claim 50 is directed to a method for reproducing coniferous somatic embryos by somatic embryogenesis (see original claim 1). The method includes growing an immature embryogenic culture derived from an explant on a nutrient medium selected from the group consisting of induction medium, maintenance medium and prematuration medium (original claim 1, specification at page 1, lines 25-28, and page 3, lines 10-13). The nutrient medium comprises

lactose (see original claim 4 and the specification at page 7, lines 5-9). The induction medium is used to induce an explant to form an embryogenic tissue (see specification at page 7, lines 1-5). The maintenance medium is used to grow and maintain the embryogenic culture (see specification at page 4, lines 10-12 and page 8, lines 3-7). The prematuration medium is used to prepare the embryogenic culture for transfer to maturation medium and subsequent development of mature embryos capable of germination (see specification at page 4, lines 7-9; page 3, lines 19-25 and the examples). The coniferous somatic embryos are *Pinus taeda* somatic embryos or hybrids thereof (original claim 9 and page 6, line 17). The maturation medium used to develop the embryos does not contain any auxin or cytokinin (see specification at page 5, lines 11-13, page 8, line 23-page 9, line 1; page 9, lines 15-19 and page 12, lines 3-5).

Claim 55 is directed to a method for reproducing coniferous somatic embryos by somatic embryogenesis (see original claim 1). The method includes growing an immature embryogenic culture derived from an explant on a nutrient medium selected from the group consisting of maintenance medium and prematuration medium (original claim 1, specification at page 1, lines 25-28, and page 3, lines 10-13). The nutrient medium comprises a galactose-containing sugar and an additional sugar (see original claims 1 and 15 and the specification at page 7, lines 5-9). The maintenance medium is used to grow and maintain the embryogenic culture (see specification at page 4, lines 10-12 and page 8, lines 3-7). The prematuration medium is used to prepare the embryogenic culture for transfer to maturation medium and subsequent development of mature embryos capable of germination (see specification at page 4, lines 7-9; page 3, lines 19-25 and the examples). The coniferous somatic embryos are *Pinus taeda* or hybrids thereof, *Pinus radiata* or hybrids thereof or *Pseudotsuga menziesii* or hybrids thereof (original claims 9-11 and the specification at page 6, line 16-18). The maturation medium used to develop the embryos does not contain any auxin or cytokinin (see specification at page 5, lines 11-13, page 8, line 23-page 9, line 1; page 9, lines 15-19 and page 12, lines 3-5).

The methods allow for higher numbers of genotypes to be successfully propagated and cryostored, results in higher numbers of seedlings and produces the seedlings at lower cost due to the greater number of mature embryos per culture (see specification at page 6, lines 20-25).

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issues presented for consideration by the Board are as follows:

1. Whether the Examiner has established a legally sufficient case of obviousness under 35 U.S.C. §103(a) over Handley (U.S. Patent No. 5,491,090) in view of Schuller (Plant Cell 60:23-31(2000) and further in view of Find (U.S. Patent No. 6, 897, 065) with respect to claims 1, 5-6, 13-14, 16-22 and 43.

2. Whether the Examiner has established a legally sufficient case of obviousness under 35 U.S.C. §103(a) over Handley in view of Fan (U.S. Patent No. 6,689,609) with respect to claims 50 and 52-54.

3. Whether the Examiner has established a legally sufficient case of obviousness under 35 U.S.C. §103(a) over Handley in view of Pullman (U.S. Patent No. 6,492,174) with respect to claims 55-63.

ARGUMENT

Rejections Under 35 U.S.C. § 103(a)

Appellants contend that the rejections of record, set forth in the final Office Action dated February 11, 2009 and the Advisory Action dated July 28, 2009 are improper.

Rejections of claims 1, 5-6, 13-14, 16-22 and 43 over Handley in view of Schuller and Find.

Claims 1, 5-6, 13-14, 16-22 and 43 were rejected as unpatentable over Handley (U.S. Patent No. 5,491,090) in view of Schuller (Plant Cell 60:23-31 (2000)) and further in view of Find (U.S. Patent No. 6,897,065). For the purposes of this rejection under 35 U.S.C. 103(a), claims 1, 6, 13, 14, 16, 17, 19, 22 and 43 stand or fall together. In addition to the arguments presented for claims 1, 6, 13, 14, 16, 17, 19, 22 and 43, additional arguments will be made in support of claims 5, 18, 20 and 21.

The Examiner contends that Handley teaches a method of regenerating *Pinus taeda* using an induction medium and a maintenance medium comprising glucose, maltose, sucrose, melezitose and a combination thereof. The Examiner acknowledges that Handley does not teach or suggest use of lactose. The Examiner alleges that Schuller teaches use of lactose in the prematuration medium and use of a combination of lactose and sucrose in the maturation medium of *Abies Alba* embryos and that Find teaches that suitable carbon sources for maturation of conifers include sucrose, maltose, lactose, fructose, glucose, maltotriose, starch, galactose, etc. The Examiner then argues that it would have been obvious to one of skill in the art to use a combination of sugars, including lactose, in the induction, maintenance, or prematuration medium. The Examiner also states that those skilled in the art would be motivated to combine the references because conifers are an important timber crop.

The Examiner has not established a* prima facie *case of obviousness.

The combination of these references does not teach or suggest each element of claims 1 or 43 or any claim dependent therefrom. Specifically, none of the references teach or suggest use of lactose and an additional sugar in an induction, maintenance or prematuration media for *Pinus taeda* somatic embryogenesis. Handley mentions several sugars as useful in the culture of somatic embryos, but does not mention galactose-containing sugars or lactose at all. Of the three references combined by the Examiner, Handley is the only one drawn to *Pinus taeda* somatic embryogenesis induction and maintenance. The absence of galactose-containing sugars or lactose from Handley supports the position that it was not obvious to use lactose and an additional sugar during any of induction, maintenance or prematuration for *Pinus taeda*. Handley only mentions use of combinations of sugars, but does not exemplify any particular combinations.

The Examiner then combines Handley with Find. Find teaches use of lactose in the maturation step of conifer somatic embryogenesis, not in induction, maintenance or prematuration. The Examiner argues that a demonstration of use of a carbon source during one phase of growth is equivalent to use of that sugar for any purpose even if the cellular process involved is completely unrelated. As detailed in the declarations of Attree and Fowke, those skilled in the art would expect the media used during different phases of growth to be different. See Fowke Declaration at 9 and Attree Declaration at 8. During induction, maintenance and prematuration the somatic embryos need to grow and be maintained in an immature state. Maturation requires the somatic embryos to stop proliferating and to differentiate into mature embryos. Thus, Find's teaching of the use of lactose in maturation media would not combine with Handley to teach the elements of the claim because Handley does not teach use of lactose in

induction, maintenance or prematuration media and those skilled in the art understand the very different media requirements for the maturation step as opposed to the induction, maintenance and prematuration steps of somatic embryogenesis.

The Examiner then combines Handley and Find with the teachings of Schuller. Schuller teaches a nutrient medium for use in *Abies alba*. As noted in the specification at page 7, lines 12-15, *Abies alba* has distinct growth requirements from other conifers. The cited references also note that *Abies* somatic embryogenesis is distinct from that of other conifers including *Pinus*. See Schuller at page 23, Introduction ("[s]omatic embryogenesis in *Abies alba*... is different from that in other conifers since the induction and proliferation of ESM could be achieved on media supplemented with cytokinin only. With sucrose, a carbohydrate source which is widely used for propagation of other conifers, no maturation of SE was observed in *Abies alba*.") and Find at col. 9, lines 10-14. Thus, one skilled in the art would not look to *Abies alba* somatic embryogenesis for direction on *Pinus taeda* somatic embryogenesis. In addition, the results of Schuller demonstrate that in most cases no late cotyledonary stage embryos were obtained and Schuller is silent as to whether any of the embryos were capable of germination as required in the instant claims.

The Examiner's reliance on the fact that conifers are an important timber crop to provide motivation to combine these references is misplaced. This fact only provides motivation to experiment in this general area. In fact, this same statement could be made about any commercially relevant technology. This requirement was recently reiterated by the Supreme Court which stated that: "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.'" *KSR v. Teleflex*, 82 USPQ2d at 1396 quoting *In re*

Kahn, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006). The Examiner has not provided adequate rationale for combining these references.

To summarize, Handley teaches only use of combinations of sugars excluding lactose in the induction, maintenance or prematuration media of *Pinus taeda* somatic embryo cultures. Find teaches use of combinations of sugars, one of which may be lactose in maturation of conifers, but fails to demonstrate the use of this sugar in any of the stages of somatic embryogenesis that require proliferation of the somatic embryos. Schuller indicates that lactose may be used as a sugar in prematuration medium of *Abies alba*, but does not produce embryos capable of germination. Thus, the combination of these references fails to teach or suggest methods of producing *Pinus taeda* embryos capable of germination using an induction, maintenance or prematuration media comprising lactose and an additional sugar.

No reasonable expectation of success in combining the references

Schuller and Handley teach away from use of lactose

Schuller teaches the use of lactose in prematuration media during *Abies alba* somatic embryogenesis. Notably as shown in Table 5 use of lactose produced no late stage cotyledonary embryos capable of germination. Schuller also indicates that use of lactose in the prematuration media produced browned embryos, but did allow for differentiation. See page 28, column 1. The discussion section of Schuller also indicates that lactose is acting to promote differentiation by causing a transient nutrient deficiency. See page 29, column 2. As indicated in the declarations of Attree and Fowke, induction, maintenance and prematuration are all phases of somatic embryogenesis in which differentiation of the cells is not desired. Thus, one of skill in the art with Schuller in hand would not have chosen to use lactose in a media for proliferation of somatic embryos as recited in claims 1, 43 and each claim dependent therefrom.

The combination was not obvious to try

The Examiner also contends that based on the combined teachings of Handley, Find and Schuller, the invention as claimed would be obvious to try. The obvious to try analysis requires that the Examiner establish that the Appellants were merely choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success. For the claims at issue, a reasonable expectation of success indicates that the references must provide those of skill in the art with a reasonable expectation of producing somatic embryos of *Pinus taeda* capable of germination and formation of plants.

In performing the analysis under the obvious to try standard the Federal Circuit has recently provided some additional guidance. In *Bayer Schering Pharma AG v. Barr Labs., Inc.*, No. 2002-1282 (Fed. Cir. August 5, 2009) and in *In re Kubin*, No. 2008-1184 (Fed. Cir. April 3, 2009), the Federal Circuit pointed to *In re O'Farrell*, 853 F.2d 894 (Fed. Cir. 1988) for guidance. As stated in *Bayer*:

> First, an invention would not have been obvious to try when the inventor would have had to try all possibilities in a field unreduced by direction of the prior art. When "what would have been 'obvious to try' would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful" an invention would not have been obvious. O'Farrell, 853 F.2d at 903. This is another way to express the KSR prong requiring the field of search to be among a "finite number of identified" solutions. 550 U.S. at 421; see also Procter & Gamble, 566 F.3d at 996; Kubin, 561 F.3d at 1359.

Bayer slip op. at 9. The Federal Circuit's analysis of obviousness is consistent with that of the Supreme Court in *KSR* in that both require a finite number of identified solutions in the prior art to support an obviousness rejection.

The cited references do not provide a finite number of identified solutions and thus do not support an obviousness rejection of the claims. Handley teaches only that glucose, maltose, sucrose, melezitose and combinations thereof can be used in induction and maintenance cultures for somatic embryogenesis. Lactose is not taught or suggested in Handley. Find teaches a list of sugars for use in maturation media (not induction, maintenance or prematuration) which includes, in addition to those listed in Handley, lactose, fructose, maltotriose, starch, galactose, etc. As described in the declarations of Attree and Fowke and as argued above, one of skill in the art would not expect media used in maturation to be useful in identifying solutions for induction, maintenance and prematuration media. Schuller teaches the use of lactose in prematuration media with *Abies alba*, but is unsuccessful in obtaining any late stage cotyledonary embryos capable of germination. Thus, Schuller does not provide a solution to the problem of providing a media for production of embryos capable of germination. The combined references teach more than nine separate sugars, each of which may be used in any combination with the other sugars for a total of 81 combinations. In addition, the cited references provide no indication of which parameters were critical to developing somatic embryos capable of germination. For example, none of the references indicate that the carbon source used is critical to development of somatic embryos capable of germination. In fact, Handley is focused on the use of activated charcoal in the media (Handley at col. 5, lines 26-29) and Find suggests that skilled person has "an infinite number of possibilities" when considering choices such as the carbon source (Find at col. 4, lines 1-3). These references provide no indication that the choice of carbon source is critical or provide any direction regarding which carbon sources are likely to be successful. Schuller provides some indication that carbon source may play a role, but completely fails to provide any direction as to which carbon sources are likely to provide

embryos capable of germination. A broad invitation to experiment in a general field cannot support an obviousness rejection.

The art of somatic embryogenesis is not predictable

"A rationale to support a conclusion that a claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded nothing more than predictable results to one of ordinary skill in the art." MPEP 2143.02 *citing KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385, 1395 (2007). As noted by the court in *KSR*, predictability in the art is required to support a conclusion of obviousness over a combination of references.

The art of somatic embryogenesis is not a predictable art. The references cited by the Examiner demonstrate that the art of somatic embryogenesis was not predictable at the time of the invention. As stated in the background section of Handley, "those working in conifer somatic embryogenesis have found that there is a striking difference between Picea conifers and Pinus conifers as to the ease with which somatic embryogenesis can be induced and plants regenerated." See Handley at col. 2, lines 20-24. Handley goes on to note that Pinus somatic embryogenesis and regeneration of plants has proved difficult. See Handley at col. 2, lines 24-31 and col. 2, line 65-col. 3, line 32. In fact, Handley notes that with the one possible exception of *Pinus caribaea,* in which one paper reported generation of only two plants with phenotypic abnormalities, no stage 3 somatic embryos capable of germination have been successfully produced in the genus Pinus. Handley at col. 4, lines 36-44. Find further supports the unpredictability and difficulties associated with Pinus somatic embryogenesis. See Find at col. 3, lines 27-30. Because the art of Pinus somatic embryogenesis is highly unpredictable, the

threshold for demonstrating obviousness of the claims is also high. Here the Examiner has not met the burden to demonstrate that one of skill in the art, in such an unpredictable field, would have found it obvious to make the combination suggested by the Examiner from the large number of possible solutions.

Unexpected results further support non-obviousness of claims 5, 18, 20 and 21

Claims 5, 18, 20 and 21 further limit the amount of lactose required in the media. None of the references teach or suggest the claimed percentages of lactose for use in producing somatic embryos capable of germination. As noted in the Declarations of Attree and Fowke, the number of mature somatic embryos capable of germination obtained using the methods claimed herein were surprising and unexpected. See Attree Declaration at 12-13 and Fowke Declaration at 11. These results were demonstrated in the Examples. This represents a significant improvement in the field because maintenance and bulk-up of tissues is a large expense and by generating higher numbers of embryos per gram of tissue the costs of somatic embryogenesis can be decreased significantly. The unexpected benefits of using lactose as compared to other more traditionally used sugars, and specifically the use of lactose at the amounts claimed, were noted in the specification at least at page 6, lines 23-25 and page 8, lines 15-21 and are noted in the Declarations of Attree and Fowke.

Rejections of claims 50 and 52-54 over Handley in view of Fan.

Claims 50 and 52-54 remain rejected under 35 U.S.C. § 103(a) as unpatentable over Handley in view of Fan (U.S. Patent No. 6,689,609). For the purposes of this rejection under 35 U.S.C. 103(a), claims 50 and 52-54 will be argued separately.

As noted previously, the Examiner withdrew the rejection of these same claims by Fan in view of Handley in an earlier Office Action. The Examiner now contends that it would have been obvious to combine the teachings of Handley, as discussed above, with Fan, which teaches use of lactose during germination, to arrive at the claimed invention. The Examiner states that it would have been obvious to try lactose in early stages of embryogenesis because it had been used on mature embryos during germination and that choice of sugar source in media is a choice of experimental design.

The Examiner has not established a* prima facie *case of obviousness.

Neither of the references teaches or suggests use of lactose in an induction, maintenance or prematuration media for *Pinus taeda* somatic embryogenesis as claimed in claims 50 and 52-54. Handley mentions several sugars as useful in culture of somatic embryos, but does not mention lactose at all. The absence of lactose from the list of sugars provided in Handley supports the position that it was not obvious to use lactose during any of induction, maintenance or prematuration. Fan does not supplement this deficiency. Fan relates to germination, not induction, maintenance or prematuration. Neither Fan nor Handley provide any teaching or suggestion that carbon sources useful in germination would also be useful for induction, prematuration or maintenance. Thus, the combination of Handley and Fan does not teach or suggest all the elements of claim 50 or any claim dependent therefrom.

The choice of lactose was not simply an experimental design choice or obvious to try

As discussed above, an analysis by the Examiner that an invention was obvious to try requires that the prior art teach a limited number of identified alternatives. A variety of potential

carbon sources exist and each can be used in combination with any other or alone. Thus, choice of a carbon source for a particular stage of growth and differentiation is not simply an experimental design choice and does not result in predictable results. As demonstrated in the examples section of the present application, the selection of carbon sources at each step of the somatic embryogenesis process leads to very different results. Consider, for example, the results in Table 5 of the specification at page 16, which demonstrate that when lactose was used as the carbon source during maintenance and prematuration of loblolly pine somatic embryos, the number of mature somatic embryos per gram of total tissue was increased 10 to 100 fold over sucrose or maltose, the sugars most commonly used in the prior art. This improvement was unpredictable and unexpected in light of the teachings of Handley and Fan, which treat all carbon sources as equivalent (as does the Examiner) and the claims are commensurate in scope with this finding.

No reasonable expectation of success in combining the references

As discussed above, and as noted in the Declarations of Attree and Fowke, those skilled in the art would expect the media used during different phases of growth to be different. See Fowke Declaration at 9 and Attree Declaration at 8. Those skilled in the art of conifer somatic embryogenesis understand that the distinct developmental phases require distinct media. The Examiner has suggested that the differences in growth hormones account for the distinct developmental effects of the media. While the Examiner may be suggesting that those skilled in the art at the time of the invention believed that alterations of growth hormones were solely responsible for the different phases of growth of the embryos, the present specification and Examples demonstrate that differences in the carbon source are important to forming embryos

capable of germination. The growth requirements (and carbon sources required) during induction, maintenance and prematuration, where cells must be maintained in a de-differentiated state and be supported for replication, are distinct from those in maturation and germination, in which cells must be allowed to properly differentiate and to stop replication. The present specification demonstrates that the carbon source used, rather than just the growth hormones, is a key factor in developing embryos capable of germination. If the Examiner's contention were true then no distinction between various carbon sources would be expected. The Examples provided in the instant specification, as well as the cited art, teach differently.

Unexpected results further support the non-obviousness of claims 52-54

Claims 52-54 further limit the amount of lactose required in the media. None of the references teach or suggest the percentages of lactose claimed in claims 52-54 for use in producing somatic embryos capable of germination. The Examiner notes in the Advisory Action, that Appellants arguments regarding unexpected results were unpersuasive due to the lack of evidence of greater than expected results from the prior art. To the contrary, Example 1 shows a greater than 4 fold increase in induction by simply changing from sucrose to lactose in the induction medium; Example 3 compares maintenance and prematuration on media containing sucrose, maltose or lactose and lactose is shown to produce over 3 times as many embryos per gram compared to sucrose and almost 2 times more embryos per gram than maltose. It is unclear to Appellants how this direct comparison between media comprising distinct sugars, including sugars disclosed in Handley, is not a persuasive showing of unexpected results. These results would not have been expected based on the teachings of Handley and Fan, which equate use of various sugars. The examples demonstrate much more than equivocal results with lactose as

compared to other sugars. Claims 52-54 are also commensurate in scope with the Examples provided in the specification. Appellants respectfully request withdrawal of the rejection and allowance of the claims.

Rejections of claims 55-63 over Handley in view of Pullman.

Claims 55-63 were rejected under 35 U.S.C. § 103(a) as unpatentable over Handley in view of Pullman (U.S. Patent No. 6,492,174). For the purposes of this rejection under 35 U.S.C. 103(a), claims 55-63 stand or fall together.

The Examiner alleges that Handley teaches use of a combination of sugars in maintenance and prematuration media. The Examiner acknowledges that Handley does not teach use of a galactose-containing sugar. The Examiner alleges that Pullman teaches initiation of *Pseudotsuga menziesii* and *Pinus radiata* embryogenic cultures in media containing 1-1.5% maltose, glucose, fructose, sucrose, galactose or a combination thereof. The Examiner then alleges that it would have been obvious to one of skill in the art to reproduce coniferous somatic embryos in maintenance or prematuration medium containing two sugars as taught by Handley and to modify the sugars by using galactose as the primary sugar as taught by Pullman.

The Examiner has not established a* prima facie *case of obviousness.

Handley is discussed in detail above and fails to teach or suggest use of a galactose-containing sugar in any media. Pullman fails to cure the deficiencies of Handley because Pullman fails to teach or suggest media for use in maintenance or prematuration and is limited to media for improving initiation (induction).

No reasonable expectation of success in the combination

One of skill in the art with the teachings of Handley and Pullman would not have expected a combination of a galactose-containing sugar and an additional sugar in the maintenance or prematuration steps of somatic embryogenesis to be useful, much less that such a combination would produce such superior results. As discussed above, the use of a galactose-containing sugar and an additional sugar in the maintenance and prematuration stages yielded unexpected results which could not have been predicted from the teachings of Handley and Pullman. For example as shown in Example 10 (page 24), the number of mature somatic embryos per gram of tissue was doubled when galactose was used in combination with an additional sugar for maintenance and prematuration of Douglas fir as compared to sucrose and maltose. Such a large increase is clearly not predictable from the combination of Handley and Pullman. In addition, the Examiner fails to note the other combinations of a galactose-containing sugar with an additional sugar in the Examples, such as Example 2 (1.5% lactose with 0.025% glucose or 0.5% sucrose). Claims 56-63 all depend from claim 55 and are not obvious over the combination of Handley and Pullman for at least the same reasons as stated for claim 55.

CONCLUSION

For the foregoing reasons, claims 1, 5-6, 13-14, 16-22, 43, 50 and 52-63 should be allowed. Appellants respectfully request that the Board reverse the rejections and pass the application to allowance.

Respectfully submitted,

Date: 2/16/10

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CLAIMS APPENDIX

1. (Previously presented) A method for reproducing coniferous somatic embryos by somatic embryogenesis comprising growing an immature embryogenic culture derived from an explant on a nutrient medium selected from the group consisting of induction medium, maintenance medium and prematuration medium, wherein the nutrient medium comprises lactose and an additional sugar, wherein the induction medium is used to induce an explant to form an embryogenic tissue, the maintenance medium is used to grow and maintain the embryogenic culture and the prematuration medium is used to prepare the embryogenic culture for transfer to maturation medium and subsequent development of mature embryos capable of germination, wherein the coniferous somatic embryos are *Pinus taeda* somatic embryos or hybrids thereof, and wherein the maturation medium does not contain auxin or cytokinin.

2-4. (Cancelled)

5. (Previously presented) The method of claim 1, wherein lactose is less than 6.0 % of the nutrient medium.

6. (Previously presented) The method of claim 1, wherein the nutrient medium is gelled or liquid.

7.-12. (Cancelled)

13. (Previously presented) The method of claim 1, wherein the prematuration medium contains less auxin and less cytokinin than the maintenance medium.

14. (Previously presented) The method of claim 1, wherein the prematuration medium further comprises abscisic acid.

15. (Cancelled)

16. (Previously presented) The method of claim 1, wherein the additional sugars are readily metabolized.

17. (Original) The method of claim 16, wherein the additional sugars are selected from the group consisting of sucrose, glucose, and fructose.

18. (Previously presented) The method of claim 1, wherein lactose is more than 1.0% of the nutrient medium.

19. (Previously presented) The method of claim 1, wherein the embryogenic culture contains early stage embryos.

20. (Previously presented) The method of claim 1, wherein lactose is less than 2.0% of the nutrient medium.

21. (Previously presented) The method of claim 1, wherein lactose is between 1.0% and 6.0% of the nutrient medium.

22. (Previously presented) The method of claim 1, wherein the nutrient medium further comprises an auxin and a cytokinin.

23.-42. (Cancelled)

43. (Previously presented) A method for reproducing conifers by somatic embryogenesis which comprises: growing *Pinus taeda* conifer cells on a nutrient medium comprising lactose, an additional sugar, an auxin, and a cytokinin to produce an immature embryogenic culture and transferring the embryogenic culture to maturation medium to obtain mature embryos capable of germination and reproduction of conifers, and wherein the maturation medium does not contain auxin or cytokinin.

44-49. (Cancelled)

50. (Previously presented) A method for reproducing coniferous somatic embryos by somatic embryogenesis comprising growing an immature embryogenic culture derived from an explant on a nutrient medium selected from the group consisting of induction medium, maintenance medium and prematuration medium, wherein the nutrient medium comprises lactose, wherein the induction medium is used to induce an explant to form an embryogenic tissue, the maintenance medium is used to grow and maintain the embryogenic culture and the prematuration medium is used to prepare the embryogenic culture for transfer to maturation medium and subsequent development of mature embryos capable of germination, wherein the somatic embryos are *Pinus taeda* somatic embryos or hybrids thereof and wherein the maturation medium does not contain auxin or cytokinin.

51. (Cancelled)

52. (Previously presented) The method of claim 50, wherein the lactose comprises 1% or more of the nutrient medium.

53. (Previously presented) The method of claim 50, wherein the lactose is between 1% and 6% of the nutrient medium.

54. (Previously presented) The method of claim 50, wherein the lactose is less than 6% of the nutrient medium.

55. (Previously presented) A method for reproducing somatic embryos by somatic embryogenesis comprising growing an immature embryogenic culture derived from an explant on a nutrient medium selected from the group consisting of maintenance medium and prematuration medium; wherein the nutrient medium comprises a galactose-containing sugar and an additional sugar; wherein the maintenance medium is used to grow and maintain the embryogenic culture and the prematuration medium is used to prepare the embryogenic culture

for transfer to maturation medium and subsequent development of mature embryos capable of germination; wherein the coniferous somatic embryo is selected from the group consisting of *Pinus taeda* or hybrids thereof, *Pinus radiata* or hybrids thereof and *Pseudotsuga menziesii* or hybrids thereof; and wherein the maturation medium does not contain auxin or cytokinin.

56. (Previously presented) The method of claim 55, wherein the coniferous somatic embryo is *Pinus radiata* or a hybrid thereof.

57. (Previously presented) The method of claim 55, wherein the coniferous somatic embryo is *Pseudotsuga menziesii* or a hybrid thereof.

58. (Previously presented) The method of claim 55, wherein the galactose-containing sugar comprises 1% or more of the nutrient medium.

59. (Previously presented) The method of claim 55, wherein the galactose-containing sugar is between 1% and 6% of the nutrient medium.

60. (Previously presented) The method of claim 55, wherein the galactose-containing sugar is less than 6% of the nutrient medium.

61. (Previously presented) The method of claim 55, wherein the galactose-containing sugar is galactose.

62. (Previously presented) The method of claim 61, wherein the somatic embryo is *Pinus radiata* or a hybrid thereof.

63. (Previously presented) The method of claim 61, wherein the somatic embryo is *Pseudotsuga menziesii* or a hybrid thereof.

EVIDENCE APPENDIX

Appended hereto are the Declarations of Dr. Attree and Dr. Fowke.

Appl. No.	:	10/764,978
Confirmation No.	:	9303
Applicant	:	Plamen Denchev
Filed	:	January 23, 2004
Title	:	METHODS FOR REPRODUCING CONIFERS BY SOMATIC EMBRYOGENESIS
IC/A.U.	:	1661
Examiner	:	Hwu, June
Docket No.	:	205502-9037-US00

I, Sally Sorensen, hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office on the date of my signature.

Sally Sorensen
Signature

May 16, 2008
Date of Signature

DECLARATION OF STEPHEN ATTREE
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Stephen Attree, do hereby declare and state the following:

1. I am currently a Director of Intellectual Property at CellFor, Inc. ("CellFor"). I have been employed by CellFor since January 1, 2000.

2. I received a Bachelors of Science Degree in Botany from the University of Manchester, UK in 1982. I received a Ph.D. in Plant Biology from the University of Manchester in 1987.

3. Attached hereto as Exhibit A is a list of my relevant patents and publications.

4. I am a co-inventor of the subject matter of all claims pending in the above-identified patent application. I make this declaration in support of prosecution of the subject application before the U.S. Patent and Trademark Office ("USPTO").

5. I have read and understand the invention as disclosed in the above-identified patent application, including the invention described by the presently pending claims.

6. I have reviewed the Office Action of January 16, 2008. I understand that claims 1, 5-9, 12-13, 16-23, 27, 28, 33-34, and 36-43 are rejected under 35 U.S.C. § 103(a) as unpatentable over Attree (U.S. Patent No. 6,627,441) in view of Handley (U.S Patent No. 5,491,090). I also understand that claims 50-54 are rejected under 35 U.S.C. § 103(a) as unpatentable over Fan (U.S. Patent No. 6,689,609) in view of Handley. I also understand that claims 55-60 are rejected under 35 U.S.C. § 103(a) as unpatentable over Coke (U.S. Patent No. 5,534,433) in view of Pullman (U.S. Patent No. 6,492,174). I believe that the evidence presented herein demonstrates that the pending claims are not obvious in light of the cited references.

7. The pending claims are drawn to methods for reproducing coniferous somatic embryos by somatic embryogenesis comprising growing an embryogenic culture derived from an explant on a nutrient medium comprising lactose, lactose and an additional sugar or a galactose-containing sugar and an additional sugar in steps prior to the maturation step, namely the induction, maintenance and/or prematuration steps.

8. Induction, maintenance and prematuration are steps prior to maturation and the media used during these steps help the conifer cells to remain undifferentiated and to proliferate. The media used during induction, maintenance and prematuration generally contain a metabolizable carbon source, hormones such as auxin and/or cytokinin and have a low osmoticum. Maturation requires the cells to slow or stop proliferating and differentiate. Maturation media generally have no auxin or cytokinin, have ABA added and have a relatively high osmoticum. Germination requires further differentiation to form seedlings. Germination media also do not contain auxin or cytokinin and generally have a low osmoticum. Induction, maintenance and prematuration require the cells to proliferate and remain in an undifferentiated state, whereas maturation and germination require the cells to stop proliferating and differentiate. Because the goals at these different steps of the process are exactly opposite, the media used at different stages of the method are, and would be expected to be, distinct.

9. U.S. Patent No. 6,627,441 to Attree relates to methods of promoting maturation of embryos by increasing the water stress on the embryos during the maturation step. See

abstract. The Examiner suggests that Attree teaches use of lactose in prematuration medium at Table 5 and column 26, lines 25-38. Attree clearly indicates that the media in Table 5 which contain lactose are maturation media and not prematuration media. See column 26, lines 26-30 ("Thus, immature somatic embryos from suspension culture were...transferred to maturation medium containing 3% sucrose, 20μM ABA and adjusted to 290mmol/kg with PEG." Emphasis added). This first maturation medium represents week 1 in Table 5 and the medium was replaced weekly during maturation with the media indicated in Table 5. Thus, lactose was first added in the third week of maturation, not during prematuration as indicated by the Examiner.

10. In addition, Attree makes clear that the lactose in the maturation media was used as an osmoticum to increase the water stress. See column 26, lines 34-35 ("water potential was increased by adding lactose"). Thus, Attree was using lactose, not as a carbon source, but instead as a means of increasing the water stress on the cells. During maturation the increased water stress reduces the moisture content of the embryos, enhances lipid storage and enhances development into mature embryos capable of germination. These effects, while being important for maturation of the embryo, are the opposite of the desired effects during induction, maintenance and prematuration of the embryos. In induction, maintenance and prematuration, a low water stress (osmoticum) is desired. Thus, the disclosure of Attree that lactose could be used as an osmoticum in the maturation medium actually discouraged the use of lactose in the induction, maintenance or prematuration stages of somatic embrogenesis.

11. Prior to the results presented in the present application, lactose, a sugar found in milk and not generally available to plants, was not believed to be metabolized by plants. The fact that lactose could be used as a carbon source was unexpected as noted in the specification at least at page 6, lines 6-9, in Example 5, page 13-14 and Example 5.1, page 14. Without the knowledge that lactose could be used as a carbon source, there would be no reason to add lactose to the induction, maintenance or prematuration media.

12. In addition, before actually doing the experiments, we would not have predicted that use of lactose in the media during induction, maintenance and/or prematuration would have

such a beneficial effect in terms of producing somatic embryos as compared to sucrose or maltose. For example, Example 1 shows over 4 fold better induction for loblolly pine in a combination of lactose and glucose than in sucrose. Example 3 demonstrates about a 2 fold increase in somatic embryos per gram of tissue when lactose was used as the sole carbon source during maintenance of loblolly pine cultures. Similar results were obtained with Radiata Pine (Examples 6 and 7).

13. The superior results obtained using these methods could not have been predicted. Even if one would have thought that lactose or galactose would be effective carbon sources for use in induction, maintenance and prematuration, the results demonstrating much higher numbers of somatic embryos per gram of tissue were surprising. This represents a significant improvement in the field because maintenance and bulk-up of tissues is a large expense and by generating higher numbers of embryos per gram of tissue the costs of somatic embryogenesis can be decreased significantly. The unexpected benefits of using a galactose-containing sugar as compared to other more traditionally used sugars were noted in the specification at least at page 6, lines 23-25 and page 8, lines 15-21. These unexpected benefits seem to be generic to conifers as all three conifers tested demonstrated a significant improvement in the number of somatic embryos produced per gram of tissue when a galactose-containing sugar was used in induction, maintenance and/or prematuration media.

14. For the reasons set forth above in paragraphs 8-13, the results demonstrated in the Examples section of the present application are surprising and would not be expected based on the cited references.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 13 May 2008

Stephen Attree, Ph.D.

Docket No.: 205502-9037-US00
Michael Best & Friedrich LLP
Two Prudential Plaza
180 North Stetson Avenue, Suite 2000
Chicago, IL 60601
(312) 222-0800

Exhibit A

Relevant Patents and Publications: Stephen Attree, Ph.D.

Issued U.S. Patents

Attree, S.M. (2003). Increasing levels of growth regulator and/or water stressing during embryo development. United States Patent 6,627,441

Attree, S.M., Fowke, L.C. (2002). Desiccation tolerant Gymnosperm embryos. United States Patent 6,372,496

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Kong, Denchev, Lobatcheva, Attree, Radley. Method of culturing conifer somatic embryos using S(+) abscisic acid. Filed 2005.

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Sutton, B, Attree, S.M., El-Kassaby, Y., Cyr, D. (2000). Clonal propagation and tree improvement using somatic embryogenesis. TAPPI Journal .

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Appl. No.	:	10/764,978
Confirmation No.	:	9303
Applicant	:	Plamen Denchev
Filed	:	January 23, 2004
Title	:	METHODS FOR REPRODUCING CONIFERS BY SOMATIC EMBRYOGENESIS
TC/A.U.	:	1661
Examiner	:	Hwu, June
Docket No.	:	205502-9037-US00

I, Sally Sorensen, hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office on the date of my signature.

Sally Sorensen
Signature

May 16, 2008
Date of Signature

DECLARATION OF LARRY FOWKE
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Larry Fowke, do hereby declare and state the following:

1. I am currently an Emeritus Professor in the Department of Biology at the University of Saskatchewan.

2. I received a Bachelors of Arts Degree in Biology from the University of Saskatchewan in 1963. I received a Ph.D. in Plant Cell Biology from the Carleton University in 1968.

3. Attached hereto as Exhibit A is a copy of my Curriculum Vitae.

4. I have read and understand the invention as disclosed in the above-identified patent application, including the invention described by the presently pending claims.

5. I have reviewed the Office Action of January 16, 2008. I understand that claims 1, 5-9, 12-13, 16-23, 27, 28, 33-34, and 36-43 are rejected under 35 U.S.C. § 103(a) as unpatentable over Attree (U.S. Patent No. 6,627,441) in view of Handley (U.S. Patent No. 5,491,090). I also understand that claims 50-54 are rejected under 35 U.S.C. § 103(a) as unpatentable over Fan (U.S. Patent No. 6,689,609) in view of Handley. I also

understand that claims 55-60 are rejected under 35 U.S.C. § 103(a) as unpatentable over Coke (U.S. Patent No. 5,534,433) in view of Pullman (U.S. Patent No. 6,492,174). I believe that the evidence presented herein demonstrates that the pending claims are not obvious in light of the cited references.

6. Embryogenesis *in vitro* is a multi-step process. First, the embryonic cultures must be induced from starting explants in an induction medium in which somatic tissue from the plant must de-differentiate and begin proliferating. The resultant cultures can be maintained, and in somatic embryogenesis reproduced, in a maintenance or proliferation medium. During this step the main goal is for the cells to proliferate as fast as possible, without developing, while maintaining the ability to mature, germinate and produce seedlings. The media used during induction and maintenance contain a relatively high level of hormones, such as auxin and cytokinin, which allow the cells to proliferate, but not differentiate. The next step, prematuration, is optional and is meant to provide a gentle transition from maintenance, where the cells proliferate, but do not differentiate, to maturation, where proliferation slows and differentiation of the cells is encouraged. The prematuration medium contains less auxin and cytokinin than the maintenance medium. The prematuration medium may also contain ABA and may have increased water stress as compared to the maintenance medium. The embryonic cultures must then be stimulated to undergo maturation by incubation in a maturation or development medium. The maturation medium does not contain any auxin or cytokinin. During maturation, the cells stop proliferating, and begin differentiating into embryos. The mature embryos can then be germinated to produce somatic seedlings. Each step in the process is defined by the media used and the effects of the media on the cells.

7. The media and culture conditions used in each of the above described steps are distinct. In particular, maturation media do not contain any auxin or cytokinin to stimulate cell growth. The induction and maintenance media contain auxin or cytokinin to stimulate cellular proliferation. The prematuration media have less auxin and cytokinin than the induction or maintenance media. In addition, maturation media have a high osmoticum and ABA added to stimulate differentiation of the cells, while induction and maintenance media have low osmoticum and no ABA, both of which are important to stimulate

cellular differentiation. Prematuration media provide a gentle transition to maturation media. Prematuration media contain some ABA and have a higher osmoticum than the induction and maintenance media.

8. The pending claims are drawn to methods for reproducing coniferous somatic embryos by somatic embryogenesis comprising growing an embryogenic culture derived from an explant on a nutrient medium comprising lactose, lactose and an additional sugar or a galactose-containing sugar and an additional sugar in steps prior to the maturation step.

9. As stated above, induction, maintenance and prematuration are steps prior to maturation and the media used during these steps help the conifer cells to remain undifferentiated and to proliferate. Maturation requires the cells to slow or stop proliferating and differentiate. Germination requires further differentiation to produce seedlings. Because the goals at the different steps of the process are exactly opposite, the media used at different stages of the method are, and would be expected to be, distinct.

10. Prior to the present application, lactose was not believed to be metabolized by conifer cells and non-metabolizable sugars would not normally be added to the induction, maintenance or prematuration media. The results presented in Example 5 of the pending application demonstrate that lactose and galactose are utilized by conifer cells. It is unexpected that lactose and galactose, sugars not generally available to plants, are metabolized by conifer cells.

11. In addition, the results presented in the Examples of the present application demonstrate that addition of galactose-containing sugars, and lactose in particular, to the induction, maintenance and/or prematuration media increased the number of somatic embryos produced per gram of tissue as compared to cells grown in the presence of other more commonly used sugars such as sucrose or maltose. It was surprising that a galactose-containing sugar could be used at all during induction, maintenance and prematuration, and even more unexpected that use of a galactose-containing sugar would produce superior results as compared to sucrose and maltose.

12. For the reasons set forth above in paragraphs 6-11, the results demonstrated in the Examples section of the present application are surprising and would not be expected based on the cited references.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: May 15, 2008

Larry Fowke, Ph.D.

Docket No.: 205502-9037-US00
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(312) 222-0800

CURRICULUM VITAE FOR LARRY FOWKE
- July 2003-

1. PERSONAL

Born June 6, 1941
Married - 3 children

2. ACADEMIC CREDENTIALS

Earned D.Sc., University of Saskatchewan, 2006
Ph.D. Carleton University, 1968, Biology Department, Plant Cell Biology
B.A. Honours, University of Saskatchewan, 1963, Biology Department

3. APPOINTMENTS AND PROMOTIONS - BIOLOGY, UNIVERSITY OF SASKATCHEWAN

Emeritus Professor, 2006-present
Professor, 1979- 2006
Head of Department, 1994-2000
Assistant Head of Department, 1992-1994
Associate Professor, 1974-1979
Assistant Professor, 1970-1974

4. SPECIAL AWARDS

ISI HighlyCited Researcher for period 1981 - 1999 (Plant and Animal Category)
Jarislowsky Chair in Biotechnology, 2002-2003.
Rawson Professor of Biology, 3 year appointment from July 2002.
Finalist (twice) for Annual Innovation Place/UST Award of Innovation, (2002 - 2005).
Distinguished Researcher Award, University of Saskatchewan, Spring 1998.
NSERC PDF, 1968-1970.

5. TEACHING

Nominated three times for University of Saskatchewan Students Union Teaching Excellence Award.

Regular teaching duties: participated in courses in general biology, cell biology, plant anatomy, plant development, electron microscope techniques.

6. INVITED SYMPOSIUM TALKS AND PLENARY LECTURES

Symposium talk, NATO Advanced Research Workshop, Kiev, Ukraine, 2002.
Symposium talk, International Association of Plant Tissue Culture & Biotechnology, Florida, USA, 2002
Symposium talk, International Union of Forest Research Organizations, Quebec, Canada, 1997.
Symposium talk, Can. Soc. Plant Physiol./Can. Bot. Assoc., Guelph, Canada, 1995.
Symposium talk, Congress on Cell and Tissue Culture, Raleigh, U.S.A., 1994.
Symposium talk, XV International Botanical Congress, Yokohama, Japan, 1993.
Symposium talk, 1st Asia-Pacific Conference on Plant Cell and Tissue Culture, Taejon, Korea, 1993.
Symposium talk, Plant Cell Biotechnologies Conference, Sofia, Bulgaria, 1993.
Symposium talk, Vesicle Traffic in Plants Conference, Gottingen, Germany, 1992.
Plenary Lecture, 8th International Protoplast Symposium, Uppsala, Sweden, 1991.

Symposium talk, Society for Experimental Botany, Warwick, England, 1990.
Symposium talk, Gordon Research Conference on Plant Cell and Tissue Culture, Plymouth, U.S.A., 1989.
Keynote talk, 4th International Conifer Tissue Culture Work Group, Saskatoon, Canada, 1988.
Symposium talk, Interkingdom Workshop on Membrane Traffic and Recycling in Eukaryotes, Wesley Chapel, Florida, 1988.
Symposium talk, XIV International Botanical Congress, Berlin, Germany, 1987.
Symposium talk, 7th International Protoplast Symposium, Wageningen, The Netherlands, 1987.
Symposium talk, NATO Advanced Study Institute, Albufeira, Portugal, 1987.
Plenary Lecture, VI International Congress of Plant Tissue and Cell Culture, Minneapolis, USA, 1986.
Symposium talk, British Society for Cell Biology, Norwich, England, 1986.
Symposium talk, Scandinavian Electron Microscope Society, Copenhagen, Denmark, 1984.
Symposium talk, 6th International Protoplast Symposium, Basel, Switzerland, 1983.
Symposium talk, V International Congress of Plant Tissue and Cell Culture, Tokyo, Japan, 1982.
Symposium talk, International Symposium for Plant Cell Culture in Plant Improvement, Calcutta, India, 1981.
Session organizer and symposium talk at XIII International Botanical Congress, Sydney, Australia, 1981.
Symposium talk, American Tissue Culture Association, Seattle, USA, 1979.
Symposium talk, International Congress for Plant Cell and Tissue Culture, Calgary, Canada, 1978.
Symposium talk, 13th Annual Electron Microscope Colloquium, Ames, USA, 1976

7. TRAINING

7 Ph.D. students, 21 postdoctoral fellows and research associates, 10 international visitors and numerous research technicians.

8. PROFESSIONAL PRACTICE

Advisory Board, Protoplasma, 1984-present
Numerous photographs published in books and review articles
NSERC grants selection committee, Cell Biology, 2003-04
Editor, Plant Cell Reports, 1997-2006
Advisory Board, Cell Biology International, 1987- 2004
Advisory Board, Plant Cell Reports, 1981-1984
Co-editor, Pro-Tem, Canadian Journal of Botany, 6 months, 1983
Associate Editor, Canadian Journal of Botany, 1979-1982
Congress Secretary, International Association of Plant Tissue Culture Congress, Calgary, 1978
Canadian Correspondent, International Association of Plant Tissue Culture, 1978-1982
Western Canadian Director, Canadian Society for Cell Biology, 1972-19, 1978-1980
NSERC grants selection committee, Cell Biology and Genetics, 1985-88, Chairman 1987-88

9. PUBLICATIONS

PAPERS IN REFEREED JOURNALS

124. J. Kang, Y. Mizukami, H. Wang, L. Fowke and N.G. Dengler. 2007 Modification of cell proliferation patterns alter leaf vein architecture in *Arabidopsis thaliana*. **Planta** 226: 1207-1218.

123. D. Bird, M. Buruiana, Y. Zhou, L. Fowke and Hong Wang. 2007. *Arabidopsis* cyclin-dependent kinase inhibitors are nuclear-localized and show different localization patterns within the nucleoplasm. **Plant Cell Reports** 26: 861-872.

122. Y. Zhou, H. Niu, F. Brandizzi, L.C. Fowke and H. Wang. 2006. Molecular control of nuclear and

subnuclear targeting of the plant CDK inhibitor ICK1 and ICK1-mediated nuclear transport of CDKA. **Plant Molecular Biology** 62: 261-278.

121. G. Pan, Y. Zhou, S. Gilmer and L.C. Fowke. 2004. An efficient method for flow cytometric analysis of pollen and detection of 2n nuclei in *Brassica napus* pollen. **Plant Cell Reports** 23: 196-202.

120. Y. Zhou, G Li, F Brandizzi, L Fowke and H Wang. 2003. The plant cyclin-dependent kinase inhibitor ICK1 has distinct functional domains for in vivo kinase inhibition, protein stability and nuclear localization. **The Plant Journal** 35: 476-489.

119. Y. Zhou, H. Wang, S. Gilmer, S. Whitwill and L.C. Fowke. 2003. Effects of co-expressing plant CDK inhibitor ICK1 and D-type cyclin genes on plant growth, cell size and ploidy in *Arabidopsis thaliana*. **Planta** 216: 604-613..

118. Y. Zhou, H. Wang and L.C. Fowke. 2002. Control of petal and pollen development by the plant cyclin-dependent kinase inhibitor ICK1 in transgenic Brassica plants. **Planta** 215: 248-257.

117. Y. Zhou, L.C. Fowke and H. Wang. 2002. Plant CDK inhibitors: studies of interactions with cell cycle regulators in the yeast two-hybrid system and functional comparisons in transgenic *Arabidopsis* plants. **Plant Cell Reports** 20: 967-975.

116. A.L Cleary, L.C. Fowke, H. Wang and P.C.L. John. 2002. The effect of ICK1, a plant cyclin-dependent kinase inhibitor, on mitosis in living plant cells. **Plant Cell Reports** 20: 814-820.

115. H. Wang, Y. Zhou, S. Gilmer, S. Whitwell and L.C. Fowke. 2000. Expression of the plant cyclin-dependent kinase inhibitor ICK1 affects cell division, plant growth and morphology. **The Plant Journal** 24: 613-623.

114. J.D.I. Harper, L.C. Fowke, S. Gilmer, R.L. Overall and J. Marc. 2000. A centrin homologue is localized across the developing cell plate in gymnosperms and angiosperms. **Protoplasma** 211:207-216.

113. H. Lui, H. Wang, C. DeLong, L.C. Fowke, W.L. Crosby and P.R. Fobert. 2000. The *Arabidopsis* Cdc-2a-interacting protein ICK2 is structurally related to ICK1 and is a potent inhibitor of cyclin-dependent kinase activity in vitro. **The Plant Journal** 21: 379-385.

112. L.C. Fowke, T. Dibbayawan, O. Schwartz, J. Harper and R. Overall. 1999. Combined immunofluorescence and field emission scanning electron microscope study of plasma membrane-associated organelles in highly vacuolated suspensor cells of white spruce somatic embryos. **Cell Biol. Internat.** 23: 389-397.

111. S. Gilmer, P. Clay, T.H. MacRae and L.C. Fowke. 1999. Tyrosinated, but not detyrosinated, α-tubulin is present in root tip cells. **Protoplasma** 210: 92-98.

110. D.A. Reid, J.N.A. Lott, S.M. Attree and L.C. Fowke. 1999. Imbibition of white spruce seeds and somatic embryos: a study of morphological changes in an environmental scanning electron microscope and potassium leakage. **In Vitro Cell. Dev. Biol. - Plant.** 35: 303-308.

109. D.A. Reid, J.N.A. Lott, S.M. Attree and L.C. Fowke. 1999. Mineral nutrition in white spruce (*Picea glauca* [Moench] Voss) seeds and somatic embryos. II. EDX analysis of globoids and Fe-rich particles. **Plant Science** 141: 19-27.

108. D.A. Reid, J.N.A. Lott, S.M. Attree and L.C. Fowke. 1999. Mineral nutrition in white spruce (*Picea glauca*

[Moench] Voss) seeds and somatic embryos. I. Phosphorus, phytic acid, potassium, magnesium, calcium, iron and zinc. **Plant Science** 141: 11-18.

107. S. Gilmer, P. Clay, T.H. MacRae and L.C. Fowke. 1999. Acetylated tubulin is found in all microtubule arrays of two species of pine. **Protoplasma**. 207: 174-185.

106. L. Kong, S.M. Attree and L.C. Fowke. 1998. Effects of polyethylene glycol and methylglyoxal bis (guanylhydrazone) on endogenous polyamine levels and somatic embryo maturation in white spruce (*Picea glauca*). **Plant Science** 133: 211-220.

105. H. Wang, Q. Qui, P. Shorr, A.J. Cutler, W.L. Crosby and L.C. Fowke. 1998. ICK1, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and cyclin D3 and its expression is induced by abscisic acid. **The Plant Journal** 17: 501-510.

104. K. Ilic-Grubor, S.M. Attree and L.C. Fowke. 1998. Comparative morphological study of zygotic and microspore-derived embryos of *Brassica napus* L. as revealed by scanning electron microscopy. **Ann. Bot.** 82: 157-165.

103. K. Ilic-Grubor, S.M. Attree and L.C. Fowke. 1998. Induction of microspore-derived embryos of *Brassica napus* L. with polyethylene glycol (PEG) as osmoticum in a low sucrose medium. **Plant Cell Reports** 17: 329-333.

102. D. Evans, P.J. Clay, S. Attree and L.C. Fowke. 1997. Visualization of Golgi apparatus in methacrylate embedded conifer embryo tissue using the monoclonal antibody JIM 84. **Cell Biol. Internat.** 21: 295-302.

101. H. Wang, L.C. Fowke and W.L. Crosby. 1997. A cyclin-dependent kinase inhibitor gene from *Arabidopsis thaliana*. **Nature** 386: 451-452.

100. L. Kong, S.M. Attree and L.C. Fowke. 1997. Changes in endogenous hormone levels during seed and zygotic embryo development in *Picea glauca* (Moench) Voss. **Physiol. Plant.** 101: 23-30.

99. P. Binarova, C. Cihalikova, J. Dolezel, S. Gilmer and L.C. Fowke. 1996. Actin distribution in somatic embryos and embryogenic protoplasts of white spruce *(Picea glauca)*. **In Vitro Cell. Dev. Biol - Plant** 32: 59-65.

98. A.R. Davis, L.C. Fowke, V.K. Sawhney and N.H. Low. 1996. Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). II.

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Quantified variability of nectary structure and function in rapid-cycling lines. **Ann. Bot.** 77: 223-234.

97. S.M. Attree, M.K. Pomeroy and L.C. Fowke. 1995. Development of white spruce [*Picea glauca* (Moench.) Voss] somatic embryos during culture with abscissic acid and osmoticum, and their tolerance to drying and frozen storage. **J. Exper. Bot.** 46: 433-439.

96. I. Leal, S. Misra, S.M. Attree and L.C. Fowke. 1995. Effect of abscissic acid, osmoticum and desiccation on 11s storage protein gene expression in somatic embryos of white spruce. **Plant Science** 106: 121-128.

95. A.R. Davis, V.K. Sawhney, L.C. Fowke and N.H. Low. 1994. Floral nectary secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). I. Nectary size and nectar carbohydrate production and composition. **Apidologie** 25: 602-614.

94. P. Binarova, P. Rennie and L.C. Fowke. 1994. Probing microtubule organizing centres with MPM-2 in dividing cells of higher plants using immunofluorescence and immunogold techniques. **Protoplasma** 180: 106-117.

93. L.C. Fowke, S.M. Attree and P.J. Rennie. 1994. Scanning electron microscopy of hydrated and desiccated mature somatic embryos and zygotic embryos of white spruce (*Picea glauca* [Moench] Voss). **Plant Cell Reports** 13: 612-618.

92. S.M. Attree, M.K. Pomeroy and L.C. Fowke. 1994. Production of vigorous, desiccation tolerant white spruce *(Picea glauca* [Moench] Voss) synthetic seeds in a bioreactor. **Plant Cell Reports** 13: 601-606.

91. S.M. Attree and L.C. Fowke. 1993. Embryogeny of gymnosperms: advances in synthetic seed technology of conifers. **Plant Cell, Tissue and Organ Culture** 35: 1-35.

90. S. Misra, S.M. Attree, I. Leal, L.C. Fowke. 1993. Effect of abscisic acid, osmoticum and desiccation on synthesis of storage proteins during the development of white spruce somatic embryos. **Ann. Bot.** 71: 11-22.

89. M. Galway, P.J. Rennie and L.C. Fowke. 1993. Ultrastructure of the endocytotic pathway in glutaraldehyde-fixed and high-pressure frozen/freeze-substituted protoplasts of white spruce (*Picea glauca*). **J. Cell Sci.** 106: 847-858.

88. L.C. Fowke. 1993. Microtubules in dividing root cells of the conifer *Pinus radiata* and the cycad *Zamia furfuracea*. **Cell Biol. Internat.** 17: 143-151.

87. T. Tautorus, H. Wang, L.C. Fowke, D.I. Dunstan, 1992. Microtubule pattern and the occurrence of pre-prophase bands in embryogenic cultures of black spruce (*Picea mariana* Mill.) and non-embryogenic cultures of jack pine (*Pinus banksiana* Lamb.). **Plant Cell Reports** 11: 419-423.

86. T.I. Baskin, C.H. Busby, L.C. Fowke, M. Sammut, F. Gubler, 1992. Improvements in immunostaining samples embedded in methacrylate: localization of microtubules and other antigens throughout developing organs in plants of diverse taxa. **Planta** 187: 405-413.

85. S.M. Attree, M.K. Pomeroy, L.C. Fowke, 1992. Manipulation of conditions for the culture of somatic embryos of white spruce for improved triacylglycerol biosynthesis and desiccation tolerance. **Planta** 187: 395-404.

84. R.M. Napier, L.C. Fowke, C. Hawes, M. Lewis, H.R.B. Pelham, 1992. Immunological evidence that plants use both HDEL and KDEL for targeting proteins to the endoplasmic reticulum. **J. Cell Science** 102: 261-271.

83. S.M. Attree, D. Moore, V.K. Sawhney, L.C. Fowke, 1991. Enhanced maturation and desiccation tolerance of white spruce (*Picea glauca* [Moench]) somatic embryos. Effects of a non-plasmolysing water stress and abscisic acid. **Ann. Bot.** 68: 519-525.

82. H. Wang, A.J. Cutler, L.C. Fowke, 1991. DNA replication and the development of preprophase bands in soybean protoplast cultures. **Physiol. Plant.** 82: 150-156.

81. H. Wang, A.J. Cutler, L.C. Fowke, 1991. Microtubule organization in cultured soybean and black spruce cells. Interphase-mitosis transition and spindle morphology. **Protoplasma** 162: 46-54.

80. H. Wang, G.P. Slater, L.C. Fowke, M. Saleem, A.J. Cutler, 1991. Comparison of cell wall regeneration on maize protoplasts isolated from leaf tissue and suspension cultured cells. **In Vitro Cell. Dev. Biol.** 27P: 70-76.

79. H. Wang, M. Saleem, L.C. Fowke, A.J. Cutler, 1991. DNA synthesis in maize mesophyll protoplasts in relation to source tissue differentiation. **J. Plant Physiol.** 138: 200-203.

78. S.M. Attree, T.E. Tautorus, D.I. Dunstan, L.C. Fowke, 1990. Somatic embryo maturation, germination, and soil establishment of plants of black and white spruce (*Picea mariana* and *Picea glauca*). **Can. J. Bot.** 68:

2583-2589.

77. L.C. Fowke, S.M. Attree, H. Wang, D. Dunstan 1990. Microtubule organization and cell division in embryogenic protoplast cultures of white spruce (*Picea glauca*). **Protoplasma** 158: 86-94.

76. T.E. Tautorus, L.C. Fowke, D.I. Dunstan. 1990. Comparative studies of protoplast development in jack pine (*Pinus banksiana* Lamb.). **Can. J. Bot.** 68: 1774-1779.

75. H. Wang, A.J. Cutler, M. Saleem, L.C. Fowke. 1990. Treatment of soybean cells with cell wall degrading enzymes inhibits nuclear division but not DNA synthesis. **J. Plant Physiol.** 135: 404-408.

74. T.E. Tautorus, S.M. Attree, L.C. Fowke, D.I. Dunstan. 1990. Somatic embryogenesis from immature and mature zygotic embryos and embryo regeneration from protoplasts in black spruce (*Picea mariana* Mill.). **Plant Science** 67: 115-124.

73. S.M. Attree, D.I. Dunstan, L.C. Fowke. 1989. Plantlet regeneration from embryogenic protoplasts of white spruce (*Picea glauca*). **Bio/Technology** 7: 1060-1062.

72. T.E. Tautorus, F. Bekkaoui, M. Pilon, R.S.S. Datla, W.L. Crosby, L.C. Fowke, D.I. Dunstan. 1989. Factors affecting transient gene expression in electroporated black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*) protoplasts. **Theor. Appl. Genet.** 78: 531-536.

71. S.M. Attree, S. Budimir, L.C. Fowke. 1989. Somatic embryogenesis and plantlet regeneration from cultured shoots and cotyledons from stored seed of black and white spruce (*Picea mariana* and *Picea glauca*). **Can. J. Bot.** 68: 30-34.

70. H. Wang, A.J. Cutler and L.C. Fowke. 1989. Preprophase bands in cultured multinucleate soybean protoplasts. **Protoplasma** 150: 110-116.

69. S.M. Attree, D.I. Dunstan and L.C. Fowke. 1989. Initiation of embryogenic callus and suspension cultures, and improved embryo regeneration from protoplasts of white spruce (*Picea glauca*). **Can. J. Bot.** 67: 1790-1795.

68. H. Wang, A.J. Cutler, M. Saleem and L.C. Fowke. 1989. Immunocytochemical detection of DNA synthesis in plant cells. **J. Plant Physiol.** 135: 15-20.

67. H. Wang, A.J. Cutler, M. Saleem and L.C. Fowke. 1989. Microtubules in maize protoplasts derived from cell suspension cultures: effect of calcium and magnesium ions. **Europ. J. Cell Biol.** 49: 80-86.

66. L.C. Fowke, M.A. Tanchak and P.J. Rennie. 1989. Serial

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section analysis of coated pits and coated vesicles in soybean protoplasts. **Cell Biol. Internat. Rep.** 13: 419-425.

65. H. Wang, A.J. Cutler and L.C. Fowke. 1989. High frequencies of preprophase bands in soybean protoplast cultures. **J. Cell Sci.** 92: 575-580.

64. H. Wang, A.J. Cutler, M. Saleem and L.C. Fowke. 1989. Microtubules in maize leaf protoplasts in relation to donor tissue and in vitro culture. **Protoplasma** 150: 48-53.

63. L.R. Griffing, L.C. Fowke and F. Constabel. 1989. Radioimmunoassay of morphinan alkaloids in *Papaver somniferum* hypocotyls, callus, and suspension cultures. **J. Plant Physiol.** 134: 645-650.

62. H. Wang, A.J. Cutler, M. Saleem and L.C. Fowke. 1989. DNA replication in maize leaf protoplasts. **Plant Cell, Organ and Tissue Culture** 48: 33-46.

61. K.M. Thai, S. Jana and L.C. Fowke. 1989. Cell membrane permeability and ultrastructural effects of difenzoquat on wild oats (*Avena fatua*). **Weed Science** 37: 98-106.

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56. I. Hakman, P. Rennie and L. Fowke. 1987. A light and electron microscope study of *Picea glauca* (white spruce) somatic embryos. **Protoplasma** 140: 100-109.

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RELATED PROCEEDINGS APPENDIX

None.